IMPROVED APPARATUS AND METHOD FOR ABSORBANCE DETECTION

The present invention concerns the field of absorbance detection. Specifically,
5   the present invention concerns an apparatus and method for absorbance
detection in instrumental situations which have short absorption path lengths.
More specifically, the present invention relates to an analytical method and
apparatus that employs a lab-on-a-chip device such as electrophoretic or flow
injection type microchips.

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Ultraviolet and visible (UV-VIS) absorbance detection provides one of the most
general detection methods in spectrochemical analysis. UV-VIS absorbance
detection is often used with microchip basal devices. However, these devices
inherently offer shallow channel of about 10-20 microns in depth, thus limiting
15   the path length through which the UV-VIS light can travel. This results in a
detection sensitivity which is insufficient for such devices to be of practical use.

Several approaches have been tried to overcome the above mentioned
limitations. Harrison et al. (Anal. Chem. 1996, 68, 1040-1046) employed a U-
20   cell which increases path length by allowing the beam to pass in a longitudinal
direction along the flow channel. However, this cell requires insertion of optical
fibers into etched channels making it difficult to manufacture. Harrison
(Electrophoresis 2000, 21 1291-1299) also tried a multipath cell in which
lithographically fabricated aluminum mirrors above and below the flow channel
25   formed a multireflection cell. A 633 nm Helium Neon laser beam was launched
through an aperture into the cell at a slightly angle. This arrangement yielded
a 5 to 10 fold increase of effective optical path length. However, this type of
device only works with a collimated laser beams. Thus, this approach is
unsuited for incoherent lamp light illumination such as is used in UV-VIS

absorbance detection methods.

A further method for improving detection sensitivity in microchip devices is disclosed in European patent application EP 0 840 113 A2, which teaches a method and means for simultaneous detection of migration patterns along a channel using cylinder optics to transmit and collect light transmitted through the channel along separation passages, eventually detecting it by means of a photodiode array (PDA) oriented parallel to the channel. The increased integration time improves signal-to-noise ratio, but with the drawback of increased analysis time.

It is therefore an object of the present invention to provide an apparatus and method for absorbance detection which overcomes the shortcomings as stated above. It is a further object of the present invention to improve the detection limit of UV-VIS absorbance detection in microchip instrumentation.

According to the present invention there is provided an apparatus for measuring absorbance comprising a light source emitting a sample beam which is incident upon a cell having a sample area, the cell being arranged to reflect the sample beam to a detector, characterized in that the apparatus further comprises a modulation means arranged to modulate the sample beam so as to improve the sensitivity of absorbance measurement.

According to an aspect of the present invention, the modulation means includes a scanning device arranged to move the sample beam from a first position in which the sample beam is incident upon said sample area to a second position in which the sample beam is incident upon the cell.

According to a further aspect of the present invention the modulation means may be a linear scanning device. The linear scanning device may be arranged

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to move the cell. Alternatively, the linear scanning device may be arranged to move an optical element.

In an alternative embodiment of the present invention, the modulation means
5   may be an angular scanning device. The angular scanning device may be a galvanometer.

Advantageously, the present invention increases the analytical sensitivity by doubling optical path length, thus doubling analytical sensitivity, and by
10 reduction of baseline noise by means of modulated or homodyne detection in combination with dual-beam detection for common mode rejection purposes. Doubling of effective optical path length is accomplished by means of reflection type sample light path, while baseline noise reduction is achieved by means of dual-beam detection together with sample modulation and synchronous
15 demodulation. Modulated detection at one certain modulation frequency, also termed homodyne detection, is a proven strategy for recovering small signals buried in asynchronous background and noise. Both homodyne detection, as well as sample modulation, can be implemented with simple circuitry, from example, dual-phase lock-in amplifiers and sample modulation means.

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In order to gain the advantages of modulated detection methods, a suitable parameter of measurement needs to be modulated in time periodically with a certain frequency. According to the present invention this is accomplished by a movement of the sample in and out of the sample light path or vice versa
25 using suitable modulation means.

Advantageously, by utilising the back reflection of light launched through the micro channel, the present invention doubles the sensitivity of the apparatus by doubling the absorption path length.

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Furthermore, by employing a modulation technique, the baseline noise is reduced, thus further improving the detection limit of the apparatus.

While the principle advantages and features of the invention have been
5   described above, a greater understanding and appreciation of the invention may be obtained by referring to the drawings and detailed description of the preferred embodiments, presented by way of example only, in which;
FIGURE 1 is a schematic illustration of a first embodiment of the present invention in which sample beam signal modulation is achieved by means of
10   linear translation of the optical element,
FIGURE 2 is a schematic illustration of a second embodiment of the present invention in which sample beam signal modulation is achieved by means of rotation of the optical element,
FIGURE 3 is a schematic illustration of a third embodiment of the present
15   invention in which sample beam signal modulation is achieved by means of translation of the microchip,
FIGURE 4 is a schematic illustration of a dual phase lock-in circuitry used for synchronous detection of the modulated absorbance signal produced by any of the embodiments shown in figures 1-3, and
20   FIGURE 5 is a schematic layout of a microchip comprising reflection mean for use in any of the embodiments shown in figures 1-3.

In figure 1 the optical setup 101 of the first embodiment of the invention is shown. Radiation 102 from light source 1, such as a Deuterium or Tungsten
25   lamp, is launched via optical means 2, such as a lens or mirror, into wavelength selecting device 3, such as a grating, bandpass filter, or monochromator. A monochromatic beam 103 emerges from the wavelength selecting device and is incident upon a double-beam in-space optical configuration 104. The use and operation of double-beam configurations is
30   well known in the art. Monochromatic beam 103 enters the double-beam

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configuration via additional optical beam steering element 4 and is incident upon semi-transparent mirror 5. A percentage of the monochromatic beam 105 is transmitted by semi-transparent mirror 5 and is incident upon optical steering element 7 which focuses the beam onto reference beam detector 9. Beam 105

5 is known in the art as the reference beam. A further percentage of the monochromatic beam 106 is reflected by semi-transparent mirror 5 and is incident upon optical steering means 10, preferably a mirror, which then reflects the beam towards movable mirror-lens assembly 13. Beam 106 is known in the art as the sample beam. Assembly 13, which preferable includes

10 a mirror 11 and optical element 12, guides the sample beam through sample area 15 of microchip 14. The sample beam then passes through the sample area and is back-reflected by reflection means 150 disposed within the microchip. The sample beam then returns along the same path through movable mirror-lens assembly 13, optical steering means 10 and again

15 encounters semi-transparent mirror 5. A percentage of the sample beam is transmitted by the semi-transparent mirror and is incident upon optical steering element 6 which focuses the sample beam onto sample beam detector 8.

In figure 1 sample beam modulation is achieved by periodically moving back

20 and forth mirror-lens assembly 13 in the directions shown by double arrow 110. This movement causes the sample beam to move from a first position (as shown in figure 1) in which the sample beam is passing through sample area 15 to a second position (to the left or right of sample area 15) in which the sample beam is no longer passing through the sample area. However, in both

25 the first and second positions, the sample beam is incident upon reflection means 150, and thus is being back-reflected as described above eventually reaching the sample beam detector 8.

Preferably, the periodic movement of assembly 13 caused by the

30 modulation means is synchronized with detectors 8 and 9. This task is

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preferably achieved by using a circuit, such as the one shown in figure 4, in which a master oscillator 507 supplies the required reference frequency for both the synchronization of the modulation means as well as for generation of sine and cosine signals by means of phase-looked-loop amplifier (PLL)

5   508 needed for demodulation. The modulated absorbance signal is generated from reference beam signal 501 and sample beam signal 502 by means of divider circuit 504 and logarithm amplifier 505. The absorbance signal is then filtered by bandpass filter 506, whose pass band is centered at modulation frequency ω. By using phase sensitive detectors 509 the

10   absorbance signal components $A_c(t)$ and $A_s(t)$ are generated by means of mixing with the respective sine or cosine signals of PLL amplifier 508. The required absorbance signal $A(t)$ eventually is obtained after rejecting sum frequency 2ω by means of low pass filters 510 and calculation of the signal magnitude by square root circuit 511.

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Further embodiments of the present invention in which variations of the modulation means are shown, are given in figures 2 and 3, where parts also appearing in figure 1 bear identical numerical designations. In figure 2 a similar optical setup 102 as shown in figure 1 is depicted, however here the

20   modulation of the sample beam is realized by varying the angle in which the sample beam is incident upon the microchip. This variation in sample beam angle can be achieved by means of a galvanometer 115 to control the movement of movable scanning mirror 116.

25   Alternatively, variation of the sample beam angle in order to achieve sample beam scanning may also be achieved by means of holographic, electro-optic, or acousto-optic scanning devices. For these variations, optical element 12 needs to be a flat-field type and produce a sample beam normal to the microchip. Preferably, this can be achieved with a telecentric scan lens or f-

30   theta lens, where the beam displacement is proportional to the scan angle, and

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the lens is positioned one focal length between the scanner device and the reflector, so that the chief ray of the sample beam is incident normal to the microchip surface.

5   In figure 3 a similar optical setup 103 as shown in figures 1 and 2 is depicted, however here the modulation of the sample beam is realized by a periodic movement of the microchip below a fixed sample beam. Microchip scanning means 130 is arranged to periodically move the microchip is the directions indicated by double arrow 131. As with the embodiment shown in figure 1, the

10 movement of the microchip must be sufficient to move the sample beam from a first position in which the sample beam passes through the sample area 15 to a second position in which the sample beam no longer passed through the sample area.

15 The periodic movement or wiggling of assembly 13 may be achieved by different modulation means, such as with a linear motor or an electrically actuated piezo device. Preferably, the linear movement of assembly 13 is in the order about 100 microns. However, the amount of movement can be varied according to the degree of modulation required.

20 The use of any of the embodiments disclosed in figures 1-3 above, advantageously allows for detection of the sample beam as well as the reference beam, thus allowing for common mode rejection of correlated fluctuations, such as light source flicker noise present in both the sample beam and in the reference beam.

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Intensity fluctuation present only in the sample beam causes noise in the sample beam signal and thus adversely effects the absorbance signal. Sample modulation decreases the noise when low frequency additive noise is the limiting noise factor. Additive noise is noise which is independent of the

30 analytical signal, such as noise which is present during on- and off-cycle of

modulation. The most common sources of additive noise are detector, amplifier, and background signal noise.

In the present invention sample modulation results in a modulated sample beam intensity $I_{s,tot}(\omega,t)$, where ω is a certain modulation frequency. In order to determine the total absorbance $A_{tot}(t)$, the quotient in Eq. 1 has to be calculated as in conventional double-beam absorbance detection from total sample signal $I_{s,tot}$, sample background signal $I_{s,Bg}$, total reference signal $I_{R,tot}$ and reference background signal $I_{R,Bg}$ according to Eq. 1.

$$A_{tot}(t) = \log\left(\frac{I_{R,tot}(t) - I_{R,Bg}}{I_{s,tot}(\omega,t) - I_{s,Bg}} \right) \qquad \text{Eq. (1)}$$

The only difference from conventional case is the fact, that $I_{s,tot}(\omega,t)$ is modulated periodically, which is indicated in Eq. (1) above by modulation frequency parameter ω.

Assuming the modulated total absorbance signal has the form shown in Eq. 2,

$$A_{tot}(t) = A_0 + A(t) \cdot \cos(\omega \cdot t + \phi) \qquad \text{Eq. (2)}$$

where A_0 and $A(t)$ are background and sample absorbance, respectively. The terms ω and ϕ are the modulation frequency and a possible phase shift relative to the phase of the modulation reference frequency. After filtering the

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absorbance signal at modulation frequency ω by bandpass filter 506, the absorbance signal is demodulated by two phase sensitive detectors (PSD) 509 by multiplication with the reference signals cos(ωt) and sin(ωt) according to Eq. 3.

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$$A_c(t) = \cos(\omega \cdot t) \cdot A(t) \cdot \cos(\omega \cdot t + \phi)$$

Eq. (3)

$$A_s(t) = \sin(\omega \cdot t) \cdot A(t) \cdot \cos(\omega \cdot t + \phi)$$

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The above sine and cosine reference signals are preferably acquired using phase-locked-loop amplifier 508 connected to modulation oscillator 507.

15 This multiplication shifts the frequency of the modulated absorbance signal, so that the output of the two PSDs 509 are given by Eq. 4.

$$A_c(t) = \frac{1}{2} \cdot A(t) \cdot \cos(2\omega \cdot t + \phi) + \frac{1}{2} \cdot A(t) \cdot \cos(\phi)$$

Eq. (4)

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$$A_s(t) = \frac{1}{2} \cdot A(t) \cdot \sin(2\omega \cdot t + \phi) + \frac{1}{2} \cdot A(t) \cdot \sin(\phi)$$

The sum frequency component at 2ω of each PSD output are then rejected by low pass filters 510 and only those frequency components within the low pass
25 filter's narrow bandwidth will pass through

For absorbance signals which are in phase with the reference signal, for example $\phi = 0$, the value of $A_c(t)$ is equal to $A(t)/2$ and the value of $A_s(t)$ will

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be zero. In general however, the phase will be non-zero and the absorbance A(t) is given by Eq. 5,

$$A(t) = 2 \cdot \sqrt{A_s(t)^2 + A_c(t)^2}$$ Eq. (5)

and is independent of the phase.

The advantage of this modulated detection technique is the fact that by appropriate selection of modulation frequency ω both signal and signal bandpass may be shifted to a "quiet" part of the noise power density spectrum, for example a spectral region where the integral noise power is lower than around $\omega = 0$.

Under detection condition, which is governed by additive non-white noise, such as noise sources showing 1/f noise behaviour like light source flicker noise or resistance fluctuation noise, a reduction results from modulated detection mode so as to allow for recovery of small signals almost completely covered by noise.

An important aspect of the invention is a dual-path cell microchip comprising suitable reflection means. Preferably the microchip is a sandwiched microchip containing reflection means in direct contact with flow channel detection volume. In figure 5 a cross section of one possible embodiment of the microchip 14 shown in figures 1-3 is shown. The glass microchip structure 14 consists of cover-plate 601 and bottom glass plate 603. The coverplate includes microstructured flow channel 603 (not shown to scale) of approximately 60 to 80 microns in width and 10 to 20 microns in depths. The

bottom plate includes high reflection means 604 positioned directly opposite the flow channel 603. Reflection means 604 is preferably a photolithographically structured metal coating of high reflectance in the spectral range of use, for example, aluminium or silver coating for UV-VIS applications. During operation the sample beam 605 passes through the flow channel 603 before backreflection by reflection means 604.

As will be appreciated by the skilled person, microstructured chips can be built on various substrates with a range of materials, and with an extensive set of techniques for fabrication of microstructures like channels. For example, glass and quartz chips are of frequent use for lab-on-a-chip devices due to a number of beneficial properties. Besides the advantageous electrical, thermal and optical properties, a large variety of surface modification methods can be easily realized.

The fabrication of channels in glass or quartz chips is usually straightforward and can be achieved with commercially available standard processes. Most commonly, the channel pattern is transferred from a photomask by photolithography to the glass substrate, spin-coated with a thin layer of photosensitive polymer (photoresist) and then exposed to UV light through the photomask. A portion of the photoresist is removed in a developing step, exposing the channel pattern for subsequent etching. The channel is etched using well known etching techniques. The reflection means is then deposited using established thin film deposition methods.

The cover plate and bottom plate are then bonded together, for example, by pressing the plates against each other at about 400 °C for several hours. During this time, atoms diffuse between the substrates forming new chemical bonds between the surfaces.